PROGRESS REPORT SUMMARY	GRANT NUMBER 5 P01 ES009581-08	
	PERIOD COVERED BY THIS REPORT	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Frank D. Gilliland, MD	FROM 11/01/04	THROUGH 10/31/05
APPLICANT ORGANIZATION University of Southern California		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Children's Environmental Health Center		

A. Human Subjects (Complete Item 6 on the Face Page)
Involvement of Human Subjects ☒ No Change Since Previous Submission ☐ Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)
Use of Vertebrate Animals ☒ No Change Since Previous Submission ☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

PROJECT NUMBER 2: POLLUTION-ENHANCED ALLERGIC INFLAMMATION AND PHASE II ENZYMES

A. SPECIFIC AIMS

There has been no change in the specific aims of this study, they are to study the role of Phase II enzymes in regulating responses to pollutants in: children's upper airways (Aim #1); the lower airways of healthy and asthmatic individuals (Aim #2) and in mechanistic animal and cellular models of allergic inflammation (Aim #3).

B. STUDIES AND RESULTS

Aim #1: We will test the hypothesis that Phase II enzyme expression in the upper airways are induced by oxidant pollutants and differ between children and adults.

Last year we developed real-time quantitative PCR (RT-PCR) to measure gene expression of sentinel Phase II enzymes. We originally intended to measure expression of 2 Phase II enzymes from cells recovered from nasal washes. However, we have now developed assays to measure 4 sentinel enzymes: GSTM1, GSTP1, NQO1 and HO-1. We have recruited 10 adult individuals and have performed a double-blind randomized cross-over exposure study to test the expression of these enzymes in response to nasal challenge with four different Diesel Exhaust Particles (DEP) doses - 0 (control), 30, 100 or 300 ug. Visits were spaced at least 4 weeks apart. All subjects had fully functional versions of GSTM1 and NQO1. The relative levels of gene expression were calculated after normalization to an internal control. In each case, control expression (that seen in cells from lavages after challenge with saline) of the genes were given an arbitrary figure of 1.0 and relative expression calculated with reference to baseline. The figure below shows that expression of NQO1 increased 24 hours after challenge with DEP. Of note is that for nearly all subjects there is a dose-response relationship between gene expression and the dose of DEP used in the nasal challenge.

Similar results were observed in response to DEP challenge in expression of GSTM1, GSTP1 and HO-1. Indeed while there was substantial inter-individual variation in the expression of any single enzyme, there was a very significant correlation between the expression of all four enzymes. That is, individuals in whom DEP challenge induced high expression of NQO1 also had high expression of each of the other genes. (see Table below)

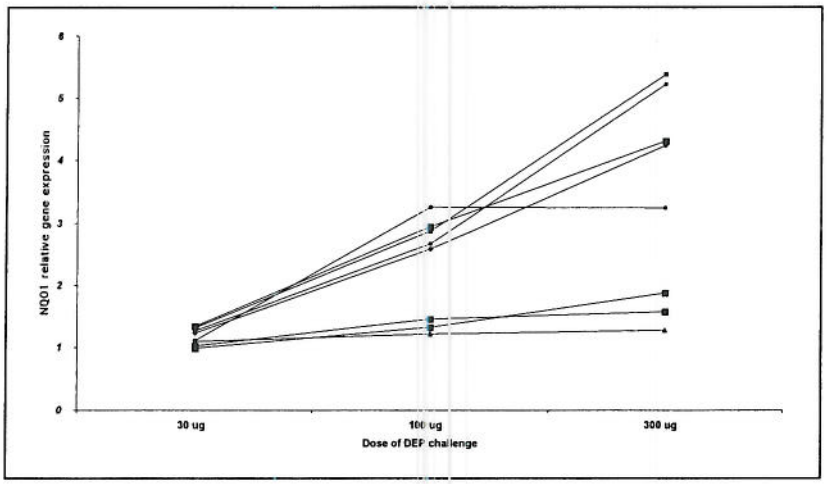

Table: *Relationship (Multiple Analysis of Variance) between expression of four Phase II enzymes in response to DEP nasal challenge (P values)*

	NQO1	GSTM1	GSTP1
GSTM1	0.031		
GSTP1	0.023	0.007	
HO-1	0.006	0.021	0.017

These results suggest that the different phase II enzymes are closely regulated. This suggests and is in agreement with several studies that indicate that DEP activates the Nrf-2 transcription factor, which controls activation of all of these genes.
Cellular inflammation was measured in nasal lavages performed 24 hours after DEP challenge. As expected there was a strong correlation between total cell number and the dose of DEP used in the nasal challenges. Moreover total cell numbers were inversely correlated with expression of GSTP1 (p=0.03) and GSTM1 (p=0.04). Measurements of other measures of cellular inflammation (e.g. chemokine and cytokine levels) are currently in progress.
In addition, we have recruited 10 children aged between 10-15 years and are performing the same procedures/exposures as with the adults. Analysis of these samples is still underway.

Aim #2: **We will test the hypothesis that Phase II enzyme expression in the *lower* airways are induced by oxidant pollutants and differ between asthmatic and non-asthmatic subjects.**
In the past year we have begun exposures of subjects to diesel exhaust to study the effect of Phase II expression on lower airway responses. To date we have performed commenced exposures on 5 healthy and 10 asthmatic subjects. As previously reported we can reproducibly produce a diesel particulate exposure level that is within 10% of the target level every time. A detailed characterization of the chemical and physical nature of the particle has been done and has been shown to be identical to diesel particles encountered in ambient real-world atmospheres. Preliminary data shows that exposure to diesel exhaust results in increase expression of all four, phase II enzymes tested in cells recovered from sputum induced 24 hours after exposure. However, it is still too early to perform any meaningful statistical analysis to compare responses between asthmatic and healthy subjects.

Aim #3: **We will determine the role of Phase II enzymes in regulating the adjuvant effects of particulate pollutants.**
We examined the responses of the airway epithelial cell line BEAS-2B and primary normal human bronchial epithelial cells upon treatment with sulforaphane a potent inducer of Nrf-2 and phase II enzymes followed by stimulation with diesel extract (0-25 ug/ml). Real-time quantitative PCR and ELISA measurements were used to assess expression of the antioxidant phase II enzymes and cytokine production, respectively. As expected, sulforaphane upregulated the expression of endogenous antioxidant enzymes in bronchial epithelial cells. Whereas diesel extract stimulated the production of IL-8, GM-CSF, and IL-1β from normal human bronchial epithelial cells, pre-treatment with sulforaphane (0-5 uM) for 24h inhibited diesel-induced cytokine production in a dose-dependent fashion. These studies suggest that sulforaphane treatment can prevent the production of pro-inflammatory cytokines in respiratory epithelial cells *in vitro*.

A. SIGNIFICANCE

Our results reinforce our belief that DEP results in the activation of the Nrf-2 transcription factor and that this results in a cytoprotective Phase II enzymes response. The level of this response may be central in determining the extent of the inflammatory response to oxidant pollutants. Increasing the body's Phase II responses either by therapeutic or dietary means appear to be promising intervention strategies for reducing the adverse effects of particulate pollutants

B. PLANS

In the next year we intend to continue recruitment of adults and children for Aims #1 and #2. We will analyze nasal lavage and sputum samples for levels of relevant chemokines and pro-inflammatory cytokines.

C. PUBLICATIONS

1. Riedl, M., **Diaz-Sanchez, D.** Biology of Diesel Exhaust Effects on Respiratory Function *J. Allergy Clin. Immunol.* 115:221-228. 2005
2. Saxon, A., **Diaz-Sanchez, D.** Air Pollution and Allergy: You are what you breathe. *Nature Immunology* 6:223-226. 2005

Sulforaphane, a phase II enzyme inducer, Inhibits Cytokine Production by Airway Epithelial Cells Stimulated with Diesel Extract. Ritz, S. and Diaz-Sanchez, D. (su